Page 6, lines 9-15:

B1

Comparison of amino acid homologies between EBV ORF BDLF1 (SEQ ID NO: 47), HSVA ORF 26 (SEQ ID NO: 46) and a 918 bp reading frame of the Kaposi's sarcoma agent which includes KS330Bam. Amino acid identity is denoted by reverse lettering. In HSVSA, ORF 26 encodes a minor capsid VP23 which is a late gene product.

Page 8, lines 6-9:

B2

Indirect immunofluorescence end point and geometric mean titers (GMT) in AIDS-KS and AIDS control sera against HBL-6 and P3H3 prior to and after adsorption with P3H3.

Page 11, lines 9-20:

B3

Representative example of IFA staining of HBL-6 with AIDS-KS patient sera and control sera from HIV-infected patients without KS. Both AIDS-KS (Figure 19A) and control (Figure 19B) sera show homogeneous staining of HBL-6 at 1:50 dilution. After adsorption with paraformaldehyde-fixed P3H3 to remove cross reacting antibodies directed against lymphocyte and EBV antigens, antibodies from AIDS-KS sera localize to HBL-6 nuclei (Figure 19C). P3H3 adsorption of control sera eliminates immunofluorescent staining of HBL-6 (Figure 19D).

Page 24, lines 24 and 25:

B4

1) AGCCGAAAGGATTCCACCAT; (SEQ ID NO: 41)
TCCGTGTTGTCTACGTCCAG (SEQ ID NO:48)

Page 24, lines 27 and 28:

B5

2) GAAATTACCCACGAGATCGC; (SEQ ID NO: 42)
AGGCAACGTCAGATGTGA (SEQ ID NO:49)

Page 24, lines 30 and 31:

B6

3) AACACGTCATGTGCAGGAGTGAC; (SEQ ID NO:43)
CGGGTGACAGTTGTGATCTAAGG (SEQ ID NO:50)

Page 26, lines 14-20:

B7

One skilled in the art may isolate and propagate the DNA herpesvirus associated with Kaposi's sarcoma (KSHV) employing the following protocol. Long-term establishment of a B lymphoid cell line infected with the KSHV from body-cavity based lymphomas (RCC-1 or HBL-6) is prepared extracting DNA from the Lymphoma tissue using standard techniques [27, 49, 66].

Page 26, lines 22-32:

B8

The KS associated herpesvirus may be isolated from the cell DNA in the following manner. An infected cell line (HBL-6 RCC-1), which can be lysed using standard methods such as hyposomatic shocking and Dounce homogenization, is first pelleted at 2000xg for 10 minutes, the supernatant is removed and centrifuged again at 10,000xg for 15 minutes to remove nuclei and organelles. The supernatant is filtered through a 0.45μ filter and centrifuged again at 100,000xg for 1 hour to pellet the virus. The virus can then be washed and centrifuged again at 100,000xg for 1 hour.

Page 101, lines 5-32:

C1
B9

The KS330Bam sequence is an internal portion of an 918 bp ORF

with 55-56% nucleotide identify to the ORF26 and BDLF1 genes of HSVSA and EBV respectively. The EBV and HSVSA translated amino acid sequences for these ORFs demonstrate extensive homology with the amino acid sequence encoded by the KS-associated 918 bp ORF (Figure 6). In HSVSA, the VP23 protein is a late structural protein involved in capsid construction. Reverse transcriptase (RT)-PCR of mRNA from a KS lesion is positive for transcribed KS330Bam mRNA and that indicates that this ORF is transcribed in KS lesions. Additional evidence for homology between the KS agent and herpesviruses comes from a comparison fo the genomic organization of other potential ORFs on the 9404 bp sequence (Figure 3A) The 5' terminus of the sequence is composed nucleotides having 66-67% nucleotide identity and 68-71% amino acid identity to corresponding regions of the major capsid protein (MCP) ORFs for both EBV and HSVSA. This putative MCP ORF of the KS agent lies immediately 5' to the BDLF1/ORF26 homolog which is a conserved orientation amount herpesvirus subfamilies for these two genes. At the 3' end of this sequence, the reading frame has strong amino acid and nucleotide homology to HSVSA ORF 27. Thus, KS-associated DNA sequences at four loci in two separate regions with homologies to gamma herpesviral genomes have been identified.

Page 135, lines 1-11:

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results were obtained by using BCBL-1 instead of HBL-6 cells, by pre-adsorbing with EBV-infected nonproducer Raji cells instead of P3H3 and by using sera from a homosexual male KS patient without HIV infection, in complete remission for KS for 9 months (HBL-6 titer 1:450, P3H3 titer 1:150). Paired sera taken 8-14 months prior to KS onset and after KS onset were available for three KS patients: KS patients 8 and 13 had eight-fold rises and patient 8 had a three-fold fall in P3H3-adsorbed BCBL-1 titers

B10 cont.

from pre-onset sera to post-KS sera.

Page 138, lines 24-39:

B11

Infections with the human herpesviruses are generally ubiquaitous in that nearly all humans are infected by early adulthood with six of the seven previously identified human herpesviruses [42]. Universal infection with EBV, for example, is the primary reason for the difficulty in clearly establishing a casual role for this virus in EBV-associated human tumors. The serologic studies identified nuclear antigen in BCBL-1 and HBL-6 which is recognized by sera from AIDS-KS patients but generally not by sera from control AIDS patients without KS after removal fo EBV-reactive antibodies. These data are consistent with PCR studies of KS and control patient lymphocytes suggesting that KSHV is not ubiquitous among adult humans, but is specifically associated with persons who develop Kaposi's sarcoma. In this respect, it appears to be

Page 147, lines 17-33:

B12

Hemophilic and Homosexual/Bisexual Male AIDS Patient Control Enrollment: Two control groups of AIDS patients were examined: 23 homosexual/bisexual men with AIDS followed until death who did not develop KS ("high risk" control group) from the Multicenter AIDS Cohort Study [16]), and 19 hemophilic men ("lowrisk" control group) enrolled from joint projects of the National Hemophilia Foundation and the Centers for Disease Controls with available follow-up information, none are known to have developed KS and <2% of hemophilic AIDS patients historically develop KS [2]. For homosexual/bisexual AIDS control patients who did not develop KS, paired PBMC specimens were available at entry into their cohort sutdy (median -35 months prior to AIDS onset) and at the study

B12 cont.

visit immendiately prior to nonKS AIDS diagnosis (median HBL-6 months prior to AIDS onset).

Page 148, lines 1-37:

B13

All samples were tested for amplifiability using primers specific for either the HLA-DQ locus (GH26/GH27) or B-globin [18]. PCR detection fo KSHV DNA was performed as previously described [7] with the following nested primer sets: No.1 outer 5'-AGCACTCGCAGGGCAGTACG-3' (SEQ ID NO:51); 5'-GACTCTTCGCTGATGAAACTGG-3' (SEQ ID NO:52); No. 1 inner 5'-TCCGTGTTGTCTACGTCCAG-3' (SEQ ID NO:53), 5'-AGCCGAAAGGATTCCACCAT-3' (SEQ ID NO:41); No. 2 outer 5'-AGGCAACGTCAGATGTTGAC-3' (SEQ ID NO:54), 5'-GAAATTACCCACGAGATCGC-3' (SEQ ID NO:42); No. 2 inner 5'-CATGGGAGTACATTGTCAGGACCTC-3' (SEQ ID NO:55), 5'-GGAATTATCTCGCAGGTTGCC-3' (SEQ ID NO:56); No. 3 outer 5'-GGCGACATTCATCAACCTCAGGG-3' (SEQ ID NO:57), 5'-ATATCATCCTGTGCGTTCACGAC-3' (SEQ ID NO:58); No. 3 inner 5'-CATGGGAGTACATTGTCAGGACCTC-3' (SEQ ID NO:55), 5'-GGAATTATCTCGCAGGTTGCC-3 (SEQ ID NO:56). The outer primer set was amplified for 35 cycles at 94^0 C for 30 seconds, 60^0 C for 1 minute and 72^0 C for 1 minute and a 5 minute final extension cycle at 72^0 C. One to three ml of the PCR product was added to the inner PCR reaction mixture and amplified for 25 additional cycles with a 5 minute final extension cycle. Primary determination of sample positivity was made with primer set No. 1 and confirmed with either primer sets 2 or 3 which amplify noneverlapping regions of the KSHV hypothetical major capsid gene. Sampling two portions of the KSHV genome decreased the likelihood of intraexperimental PCR contamination. These nested primer sets are 2-3 longs more sensitive for detecting KSHV sequences than the previously published KS3330233 PRIMERS [6] and are estimated to be able to detect <10 copies of KSHV genome